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Forum on antimicrobial resistance

The epidemiology of antibiotic resistance in *Campylobacter*

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Abstract

Antibiotic resistance, particularly with the fluoroquinolones and macrolide antibiotics, has now emerged globally with thermophilic campylobacters, including *Campylobacter jejuni* and *C. coli*, giving rise to concerns about how these organisms have acquired such resistance characteristics, as well as consequences for human and animal treatment. This review examines (i) the clinical epidemiology of antibiotic resistance in human and animal thermophilic campylobacters, (ii) an update on resistance rates globally, (iii) surveillance of antimicrobial resistance in campylobacters originating from animals, particularly poultry, (iv) the role of the environment in the acquisition and transmission of antibiotic-resistant campylobacters, as well as (v) issues of biocide resistance in campylobacters.

Keywords: Poultry; Fluoroquinolone; PCR; Pigs; Japan; Australia; New Zealand; Egypt; Hypochlorite; Biocides; Macrolide; UK; Ireland

1. Clinical perspective

The targeted selective toxicity of antimicrobial agents has ensured their widespread use to combat infection; however, it has paradoxically resulted in the emergence and dissemination of multi-drug-resistant (MDR) zoonotic bacterial pathogens [1]. Antimicrobial resistance in both medicine and agriculture is recognized by the World Health Organisation (WHO), along with other various national authorities as a major emerging problem of public health importance. It represents a significant challenge of global dimensions to human and veterinary medicines with the prospect of therapeutic failure for life-saving treatments now a reality.

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Although most *Campylobacter* infections are usually self-limiting and treated with fluid replenishment, antimicrobial therapy may be prudent for patients with severe, prolonged or systemic infections or to control infection in high-risk groups [2]. As *Campylobacter* spp. are considered to be zoonotic pathogens, antimicrobial resistance among isolates in the animal reservoir has serious implications for the treatment of campylobacterosis in humans.

Currently, macrolides and fluoroquinolones are the antimicrobial agents of choice when therapeutic intervention is warranted [3–5]. Tetracyclines have been suggested as an alternative choice in the treatment of clinical campylobacterosis, but in practice are rarely used. Intravenous aminoglycoside therapy may also be considered in more serious cases of *Campylobacter* infections, such as bacteraemia and other systemic infections [6].

The vast majority of clinical *Campylobacter* infections result in a self-limiting diarrhoeal illness. Complicated cases such as invasive disease in those with reduced immunity are uncommon as are chronic sequellae, e.g. Guillian Barré syndrome (GBS) and reactive arthritis. Epidemiological studies have examined the clinical impact of antibiotic resistance in *Campylobacter* infections. Patients with quinolone-resistant *Campylobacter jejuni*, treated with fluoroquinolones in Minnesota in 1997 had a median duration of diarrhoea of 10 days compared to 7 days in those with sensitive strains [7].

In a retrospective case comparison study, [8] five of 28 patients (31%) with ciprofloxacin-resistant *Campylobacter* were hospitalised for gastroenteritis compared to one of 31 patients (3%) with sensitive strains. However, the median age of patients in the two groups was 46 versus 24 years.

A case control study performed in 1998–1999 by a CDC based team found that in a group of 290 persons with *Campylobacter* infection who did not take antidiarrhoeal medication, those with ciprofloxacin-resistant strains had a mean duration of diarrhoea of 9 days, compared to 7 days in those with sensitive isolates ($p = 0.04$) [9]. Of 85 persons who took fluoroquinolone antimicrobials only, diarrhoea lasted a mean of 8 days in those with resistant strains but 6 days in those with sensitive isolates ($p = 0.20$). In 63 patients who took no antimicrobials, those with ciprofloxacin-resistant isolates had diarrhoea for a mean of 12 days versus 6 days for sensitive strains ($p = 0.04$). In this study, patients with sensitive or resistant *Campylobacter* were equally likely to be hospitalised. Surprisingly, persons with sensitive strains spent longer in hospital (mean 3 days) than those with resistant isolates (mean 2 days; $p = 0.01$).

The interpretation of the data from Nelson et al. [9] has been contested by a group who claim that for infections acquired in the USA an association between antimicrobial resistance and longer duration of diarrhoea is unproven [10].

A Danish study in 2001–2002 [11] showed that persons with quinolone-resistant *C. jejuni* had a median duration of illness of 13.2 days compared to 10.3 days with sensitive strains. It was not established how many of these persons had taken fluoroquinolone antimicrobials. For *Campylobacter coli* there was no difference in mean duration of illness between those with sensitive and resistant strains.

Recently, another Danish study [12] examined the relationship between infection with sensitive or resistant *Campylobacter* and adverse health events, defined as invasive disease or death within 90 days. Twenty-two of 3471 (0.6%) patients with stools positive for *Campylobacter* experienced adverse events. Those with quinolone-resistant strains acquired in Denmark had a 9.68-fold (95% CI, 2.23–42.04) increased risk of adverse events within 30 days adjusted for sex, age and co-morbidity. Infection with an erythromycin-resistant strain was associated with a 5.51-fold (95% CI, 1.19–25.5) risk of an adverse event within 90 days.

The available evidence suggests that those infected with resistant *Campylobacter* experience illness that is prolonged and more severe than those with sensitive strains. However, the magnitude of this effect is not as yet sufficiently great to require a move away from erythromycin and fluoroquinolones for the treatment of most cases of *Campylobacter* gastroenteritis. Furthermore, it is not clear whether the additional problems arise because a strain is more resistant or whether resistant strains tend to possess additional virulence factors that are not directly related to their reduced susceptibility to antimicrobials.

1.1. Antimicrobial susceptibility testing in Campylobacter *spp.*

Several laboratory methods including disc diffusion, broth microdilution, agar dilution and the Epsilometer-test (E-test) have been employed to determine the antimicrobial susceptibility of *Campylobacter* to a range of antimicrobial agents [13–17]. Although comparable standardised procedures for susceptibility testing are available for a wide range of organisms, based on the guidelines defined by the National Committee for Clinical Laboratory Standards (NCCLS), no internationally accepted criteria is available for susceptibility testing of *Campylobacter* spp. In June 2002, the NCCLS guidelines recommended agar dilution as a testing method for *Campylobacter*; however, to date the EU Community Reference Zoonosis Laboratory has not recommended a specific method for the susceptibility testing of *Campylobacter* spp. and breakpoints do not exist [5]. As a result there is a debate in the literature regarding the testing methodology and interpretation of results of antimicrobial resistance of *Campylobacter* spp. [15].

A number of studies have investigated the correlation between the methods above and results have been reported to vary only minimally. In 1997, Gaudreau and Gilbert [13] compared disc diffusion and agar dilution methods and reported complete agreement for tetracycline and ciprofloxacin, with only minor differences for erythromycin. Frediani-Wolf and Stephan [18] reported complete correlation with respect to the separation of *C. jejuni* isolates into susceptible and resistant groups independently, whether disc diffusion, E-test or microdilution broth methods were used. Furthermore these authors concluded that the disc diffusion method was a reliable and easy tool for monitoring the prevalence of resistant *C. jejuni* strains. The E-test and agar dilution methods appear to be in agreement when used on a small number of isolates

from a single geographic location but for larger collections, microdilution is the preferred protocol [16]. In contrast to these studies, Ge et al. [15] reported that the determination of resistance values can vary depending on the antimicrobial agent(s) being investigated.

The use of molecular techniques offers an alternative means for detecting antimicrobial resistance among bacterial isolates, however, this depends on a prior knowledge of the genetic basis for that resistance. Molecular techniques such as the Mismatch Amplification Mutation Assay (MAMA-PCR) [19] and the Lightcycler mutation assay [1,20] have been successful for the detection of ciprofloxacin-resistant *C. jejuni* and *C. coli* isolates, by identifying a mutation, Thr-86-Ile in the *gyrA* gene, commonly associated with ciprofloxacin resistance in *Campylobacter* spp. A line probe assay has also been developed which offers a rapid and easy method for the simultaneous detection of the mutations associated with resistance to both quinolones and macrolides in *C. jejuni* and *C. coli* isolates [21].

Undoubtedly, one of the advantages of using these methods includes the possibility of direct detection from a sample without having to culture the organism [22]. These techniques can also facilitate the analysis of organisms which are difficult to grow, and also offers the possibility of screening large numbers of organisms for specific mutations within a single assay. Major disadvantages of using these molecular techniques are that they may not detect resistance if a new, unexpected resistance mechanism is present [23], and the necessity to perform a separate assay for each antimicrobial agent tested. For these reasons, it is more beneficial to combine phenotypic and genotypic methods of susceptibility testing.

2. Surveillance of antimicrobial resistance in human clinical *Campylobacter*

2.1. Australia and New Zealand

Campylobacter infection is the most commonly notified disease in New Zealand, with 326.8 cases notified per 100,000 population in 2003 [24], but there is relatively little information available on antibiotic sensitivity. A study of 202 enteric isolates (not speciated) from Auckland [25] found that antibiotic resistance was generally uncommon, with no resistance detected to gentamicin, with 1%, 1.5% and 2.5% resistance to doxycycline, erythromycin and ciprofloxacin, respectively. Three years later, Goodchild et al. [26] in a study of 200 isolates from the same area of New Zealand reported that erythromycin resistance was more common in *C. jejuni* than in ''non-jejuni'' isolates, with 3% of 15 *C. jejuni* isolates resistant to erythromycin versus 40% of 185 ''non-jejuni'' isolates. Similarly, 4% of the *C. jejuni* isolates were resistant to ciprofloxacin against 20% resistance in ''non-jejuni'' isolates. Only one of the 200 isolates was resistant to tetracycline and there was no resistance to gentamicin. The only animal data comes from a study by Harrow et al. [27] of 251 human, animal and environmental strains from the south island of New Zealand which found no resistance to ciprofloxacin, nalidixic acid or tetracycline but five pig offal strains (one *C. jejuni* and four *C. coli*) were highly resistant to erythromycin. Tylosin (a macrolide) is commonly used in pigs for treatment and prevention of respiratory disease and may account for the erythromycin resistance seen.

Campylobacter infection is also very common in Australia with an annual notification of 116 cases per 100,000 in 2003 [28]. There is very little information in the published literature on resistance in human strains. Korolik et al. [29] reported on some studies of human strains collected between 1989–1990 and 1994–1995. There were 81 strains of *C. jejuni* and eight of *C. coli* in the first study and 79 of *C. jejuni* and six of *C. coli* in the second study. Erythromycin resistance dropped from 44% to 5% in *C. jejuni*, and from 50% to 33% in *C. coli*; doxycycline resistance fell from 10% to 2.5% in *C. jejuni* and from 25% to 16% in *C. coli*. No enrofloxacin resistance was found. In a study of 100 strains (79 *C. jejuni*, 19 *C. coli* and 2 *Campylobacter lari*) Huysman and Turnidge [30] found little resistance, with all strains sensitive to erythromycin, gentamicin, nalidixic acid and ciprofloxacin (apart from *C. lari* which showed its typical intrinsic resistance to nalidixic acid). Nine strains were resistant to tetracycline. A study in the Hunter region of NSW investigated the resistance of 180 isolates of *C. jejuni*. Eleven percent of isolates were resistant to tetracycline, 3.4% to nalidixic acid, 2.9% to ciprofloxacin, 64% to ampicillin, 3.4% to erythromycin and 48% to roxithromycin. No isolates were resistant to gentamicin. When travel history was taken into account, most of the nalidixic acid and all of the ciprofloxacin strains were acquired overseas. In the case of erythromycin resistance, all resistant strains were acquired locally but more overseas acquired strains were resistant to tetracycline and roxithromycin [31]. Unicombe et al. [32] reported that in addition to the cases reported in Sharma's study, all fluoroquinolone-resistant strains ($n = 7$) detected in two other Australian studies had been acquired overseas. An association between overseas travel and fluoroquinolone resistance in human isolates had been noted previously [33].

There are few published studies on resistance in non-human Australian isolates. Korolik et al. [29] included 88 chicken strains (79 *C. jejuni* and 9 *C. coli*) in their study. Thirty-one percent of *C. jejuni* and 22% of *C. coli* were resistant to erythromycin; 10% of *C. jejuni* and 33% of *C. coli* were resistant to doxycycline. No isolates were resistant to enrofloxacin. Two hundred and sixteen *Campylobacter* (142 *C. jejuni*, 74 *C. coli*) were isolated from chicken samples from three sources [34]. There were significant differences in resistant rates between the three sources, presumably reflecting differences in antibiotic use practices. Resistance to ampicillin in *C. jejuni* varied between 50% and 61%, to lincomycin and tylosin from 4% to 28%, to tetracycline from 15% to 37%, and erythromycin from 0% to 11%. Resistance to neomycin and gentamicin was negligible and one isolate from one source was resistant to ciprofloxacin. In the case of *C. coli*, ampicillin resistance was around 35%, resistance to tetracycline varied from 16% to 36%, to lincomycin from 4% to 30%, to

erythromycin from 0% to 17%; and to tylosin from 1% to 17%; resistance to neomycin and gentamicin was negligible and two isolates from one source were resistant to ciprofloxacin. An unpublished study of antibiotic resistance in 216 non-speciated campylobacters from pigs (R Pratt and MD Barton, personal communication) found 50% of the isolates were resistant to erythromycin, 47% resistant to clindamycin, 43% to tetracycline, 32% to ampicillin and 3% to gentamicin. No resistance to chloramphenicol or ciprofloxacin was detected. A subsequent study of carcass swabs from pigs [35] reported that 2–3% of isolates were resistant to ampicillin and neomycin, 18–24% resistant to clindamycin, 76–87% to erythromycin, 84–87% to lincomycin, 80–87% to tylosin and around 70% to tetracycline. There was no resistance to gentamicin or ciprofloxacin. Widespread use of tylosin, lincomycin and tetracyclines in animals no doubt drives the increased resistance levels seen in animal isolates.

Fluoroquinolones are not registered for use in food producing animals in Australia, and the results of testing of chickens and pigs support the view that these antibiotics have not been used in livestock. On the other hand, fluoroquinolones are often used to treat pet animals. The lack of use of fluoroquinolones in food producing animals probably accounts for the low frequency of resistance to this class of antibiotic in Australians, apart from those who acquire their infections overseas.

2.2. South Africa

The Red Cross Children's Hospital (RXH) in Cape Town, South Africa, has been isolating and characterizing *Campylobacter* spp. since 1977. Our isolation protocol utilizes membrane filtration onto antibiotic-free plates and subsequent incubation at 37 °C in a hydrogen-enriched microaerobic atmosphere, allowing excellent growth of *C. jejuni* and fastidious organisms such as *Campylobacter upsaliensis* and *Campylobacter concisus*. These organisms may be sensitive to the agents incorporated into conventional *Campylobacter* selective media, or may have an absolute requirement for hydrogen, and often cannot be isolated by conventional means. Currently ~20% of paediatric diarrhoetic stools at RXH yield *Campylobacter* spp. Only ~1/3 of the *Campylobacter* isolates from enteritis and septicaemia patients are *C. jejuni*, the rest being emergent *Campylobacter* spp. such as *C. upsaliensis* and *C. concisus*. Emergent campylobacters are infrequently isolated, and their disease potential is just beginning to be appreciated.

Over the period 1998–2005, 10,413 stools were submitted to the diagnostic microbiology laboratory at RXH. One thousand nine hundred and forty-six strains of *Campylobacter* and the related genera *Arcobacter* and *Helicobacter* were isolated from diarrhoetic paediatric stools. Antibiotic susceptibility studies were done at 37 °C by standard disc diffusion, with the following antibiotic agents tested at the given concentration: nalidixic acid (30 μg), cephalothin (30 μg), erythromycin (15 μg), ciprofloxacin (1 μg), and ceftriaxone (30 μg).

2.2.1. C. jejuni

This organism was consistently the most frequently isolated *Campylobacter* ($n = 678$) from RXH paediatric enteritis patients. Since 1998, an increasing trend in erythromycin resistance of *C. jejuni* strains has been noticed, from 3.4% to 7.2%. Ciprofloxacin resistance increased from 1.4% to 29% and nalidixic acid resistance increased from 5.7% to 41% in these same isolates. Since 2002, ceftriaxone resistance increased from 3.6% to 24.6%. For the first time, multiple antibiotic-resistant isolates, resistant to three classes of antibiotic have been seen. In 2005, a stool isolate and two blood culture isolates were resistant to ciprofloxacin, erythromycin *and* ceftriaxone.

2.2.2. *Other* Campylobacter *spp.*

C. concisus ($n = 457$) and *C. upsaliensis* ($n = 433$) were the next most frequently isolated *Campylobacter* spp. from paediatric diarrhoetic stools. Since 1998, resistance to nalidixic acid increased from 40% to 62% in *C. concisus* isolates. Ciprofloxacin resistance increased from 6.9% to 18%, and erythromycin resistance increased from 4.8% to 21.7% in these isolates. Ceftriaxone resistance remained stable at 2%. Twelve stool isolates of *C. concisus* were resistant to several classes of antibiotic, such as ciprofloxacin + erythromycin, or ciprofloxacin + ceftriaxone. *C. upsaliensis* isolates showed increasing resistance to nalidixic acid from 1.7% to 3.7%. Erythromycin resistance was constant at ~9%, while no resistance to ceftriaxone was noted. One ciprofloxacin-resistant *C. upsaliensis* isolate was recorded in 2002. The true disease potential of *C. concisus* and *C. upsaliensis* is not yet fully understood, and the pathogenicity of these organisms is probably highly underrated at present. There is some evidence that local chickens, pigs and ostriches carry antibiotic-resistant *Campylobacter* strains, but data is scanty at present, and warrants additional research.

2.3. The Middle East

Limited data are available on *Campylobacter* resistance rates from north African and middle eastern countries, even though *Campylobacter* remain a major contributor to acute enteric infections in these countries [36–38].

2.3.1. Egypt

In a study by Putnam et al. [39] on the annual prevalence of antimicrobial susceptibility among *C. jejuni* and *C. coli* recovered from rural Egyptian children from 1995 through 2000, *C. jejuni* and *C. coli* demonstrated significant decreasing trends in ciprofloxacin susceptibility over the study period. In general, *C. coli* demonstrated a higher degree of susceptibility than *C. jejuni*. There was no indication of frank macrolide resistance among any *Campylobacter*. Moreover, there were significant positive trends in both MIC_{50} and MIC_{90} values for erythromycin and azithromycin during the study period. The study demonstrated significant decrease in *Campylobacter* susceptibility from 1995 through 2000 among paediatric diarrhoea cases in rural Egypt. In another study, it was reported

that *C. jejuni* and *C. coli* were resistant to cephalothin, azetronam, and streptomycin. [40]

2.3.2. Lebanon

Talhouk et al. [41] tested different *Campylobacter* isolates from diarrhoeic human stools, as well as from chicken caeca and raw chicken carcasses. Overall, most isolates showed high to moderate susceptibility to gentamicin (97%), amoxicillin/clavulanate (95%), clindamycin (77%), chloramphenicol (77%) and ampicillin (69%). Lower susceptibility was observed against tetracycline (49%), erythromycin (47%), ciprofloxacin (39%) and norfloxacin (36%). This overall susceptibility applied to both *C. jejuni* and *C. coli*, although *C. coli* mostly showed higher susceptibility than *C. jejuni*. Beta-lactamase production was detected in 59% of all the isolates, being higher in *C. coli* (72%) than *C. jejuni* (33%).

2.3.3. Jordan

In a study on 1400 diarrhoeal Jordanian patients, of the pathogenic bacteria isolated, 0.9% was *Campylobacter*. Cotrimoxazole resistance was observed in 77% of *Campylobacter* isolates [42].

2.3.4. Iraq

The Harvard Study Team have previously reported on the effect of the Gulf crisis on children in this country [43]. In a study carried out in Northern Iraq, seven isolates of *Campylobacter* were identified from 105 stool samples from children with diarrhoea and their sensitivity results included chloramphenicol, gentamicin: 100% sensitive; erythromycin, tetracycline, nalidixic acid: 85.7% sensitive; ampicillin: 71.4% sensitive; kanamycin: 57.1% sensitive; amoxicillin: 42.8% sensitive. All isolates were resistant to cephalothin, trimethoprim and sulphamethoxazole [44].

2.3.5. Saudi Arabia

Saudi Arabia holds a unique position in that the extraordinary enmasse migration during Hajj, leads to repeated outbreaks of infectious diseases, including gastroenteritis [45]. In a 12-month survey on 1217 patients with diarrhoea carried out in Jeddah, campylobacters were isolated from 55 (4.5%) patients, second in prevalence to salmonellae (6.2%). Analysis of the results showed that 69% were *C. jejuni* and 31% *C. coli*. Resistance to erythromycin and tetracycline was observed in 7.3% and 32.7% of the isolates, respectively. [46].

2.3.6. Yemen

Banajeh et al. [47] reported that *Campylobacter* was found to be an extremely uncommon agent of childhood diarrhoea, accounting for only 1.6% of the positive cultures.

2.3.7. Kuwait

Albert et al. [48] isolated 64 *Campylobacter* strains from human diarrhoeal stools in Kuwait during 2000–2003. Approximately, 53% (34/64) of the isolates were resistant to ciprofloxacin (MIC, 4–64 μg/ml) and 5% (3/64) to erythromycin (MIC $>$ 256 μg/ml).

2.4. Israel

In a previous study by Schwartz et al. [49], 30 *C. jejuni* strains isolated from stools of Israeli children with enteritis were tested for sensitivity to eight antimicrobial agents (MIC) and the presence of plasmids. It was found that all the isolates were sensitive to ciprofloxacin, ofloxacin, furazolidone and erythromycin. Of the 30 strains tested, 21 (70%) were found to be tetracycline-resistant, a relatively high resistance rate as compared with data from other countries and previous reports from Israel. Plasmids were detected in 17 out of 30 *C. jejuni* isolates (55.6%). A total of nine different plasmid profiles could be distinguished; six profiles were represented by one strain each. Of the 21 tetracycline-resistant strains, plasmids were found in 17 isolates (80%) carrying from 1–2 to 5 plasmids of various sizes. No plasmids were found in tetracycline-sensitive strains, with the exception of one isolate which contained a 24.4 MDa plasmid and was co-trimoxazole-resistant. This study indicated a relatively high percentage of tetracycline-resistant *C. jejuni* isolates in the Tel Aviv area, with 80% of these strains harbouring various plasmid profiles.

2.5. Japan

Itoh et al. [50] first examined a total of 245 isolates of *C. jejuni* including 111 from human diarrhoeal cases in Japan which were isolated from 1979 to 1982, including 134 from cattle, poultry and wild birds, as well as 72 isolates of *C. coli*, including 18 from humans and 54 from swine for their susceptibility to nine antimicrobial agents (APC, ampicillin; EM, erythromycin; CO, chloramphenicol; TC, tetracycline; AMK, amikacin; GM, gentamicin; KM, kanamycin; CER, cephaloridine; NA, nalidixic acid). All human isolates of *C. jejuni* were susceptible to APC, EM, CP, AMK, GM, KM and NA without an isolate resistant to KM. MICs of CER were distributed between 3.12 and $>$100 μg/ml. In addition, 69% of human isolates of *C. jejuni* were resistant to TC. Although the susceptibility profile of *C. coli* against these agents were essentially the same as that of *C. jejuni*, in the isolates isolated from swine, appearance of isolates highly resistant to EM (44%) and KM (59%) was common. Moreover, the isolates resistant to multiple drugs were observed both in *C. jejuni* and *C. coli*.

When Sagara et al. [51] characterized 111 *C. jejuni* and 10 *C. coli* isolates isolated from stools of diarrhoeal patients at a Tokyo Metropolitan Hospital from 1981 to 1982 for their susceptibility to antimicrobial agents, all of the *C. jejuni* isolates were susceptible to CP, CPEX (ciprofloxacin), EM, KM and NA, but 55% were TC-resistant. In 10 *C. coli* isolates, a high prevalence of multiple antibiotic resistance was noted. In addition, 48 *C. jejuni* isolates isolated from infants and children with acute enteritis in the University Hospital, Nothern Kyushu, from 1983 to 1985 were highly susceptible to GM, AMK, KM, EM, JM (josamycin) and CP [52].

Between the second half of the 1980s and the 1990s, various studies were carried out. Tadano et al. [53] examined antimicrobial susceptibilities of 600 clinical isolates of *C. jejuni*

during a 6-year period from 1989 through 1994 in four Tokyo Metropolitan Hospitals. The overall resistant rates were as follows: NFLX (norfloxacin) (7.5%); OFLX (ofloxacin) (7.5%); CPFX (7.3%); NA (10.3%); EM (0.6%) and TC (43.2%). In addition, when six antimicrobial agents (APC, EM, TC, NA, NFLX and OFLX) were employed for the 68 isolates (*C. jejuni*, 42; *C. coli* 26) isolated from broilers in the southern part of Japan from 1995 to 1999, quinolone-resistant *Campylobacter* isolates numbered 22 (32.4%) [54]. Thus, in the second half of the 1990s, a high frequency of quinolone-resistance was found in both *C. jejuni* and *C. coli* in the veterinary field in Japan, whereas a high level of EM-resistant was found only in *C. coli* but not in *C. jejuni*. Moreover, all the isolates except one, were cross-resistant to NA, OFLX and NFLX.

In addition, when Ishihara et al.[55] performed the nationwide monitoring of antimicrobial resistance in a total of 468 *Campylobacter* isolates obtained from food producing animals on farms, during the period, 1999–2001, high frequencies of resistance to OTC (oxytetracycline) and DHSTH (dihydrostreptomycin) were observed. The frequencies of resistance in *C. coli* to AMGLY (aminoglycosides), MACD (macrolides), TC and the quinolones were higher than those in *C. jejuni* to the same drugs. All *C. jejuni* isolates were susceptible to MACD, whereas 48.4% of *C. coli* were resistant to the antibiotics. Since resistance to the fluoroquinolones was observed in *C. jejuni* isolates from broilers (12%) and layers (2.6%), the authors suggested that the level of fluoroquinolone resistance in Japan could be ranked as low.

In 2004, Niwa et al. [56] reported on antimicrobial resistance of 193 human *Campylobacter* isolates and 56 poultry meat isolates isolated in Kanagawa prefecture from 1979 to 2001 and from 1997 to 2000, respectively against NA, OFLX, EM, TC, APC, GM and PM (phosphomycin). The authors indicated that the frequency resistant to the quinolones of the human isolates increased remarkably, whereas TC-resistant isolates decreased during the study periods.

2.6. Europe

Most recently (September 2005), updates on the occurrence of antimicrobial resistance data in Europe were presented at the *13th International Workshop on Campylobacter, Helicobacter and Related Organisms*.

In a comprehensive study by Gallay et al. in France (Abstract 23; Surveillance of *Campylobacter* infection in France 2002–2004), involving up to 417 laboratories (up to 325 private laboratories and up to 92 hospital laboratories) and 4090 isolates, overall antibiotic resistance to erythromycin was less than 5%, 31% resistance to tetracycline, 39.2% resistance to ampicillin. They reported that antibiotic resistance to all antibiotics studied was higher in *C. coli* than *C. jejuni*, with 39.9% and 27.2% resistance to nalidixic acid, respectively. Resistance to gentamicin was virtually non-existent.

In a similar study in Germany, (Abstract 44; Comparative activities of fluoroquinolones and other non-related antibiotics against human *Campylobacter* spp. isolated from 1999 to 2004 in northern Germany), Krausse and Ullmann examined the activities of six fluoroquinolones (ciprofloxacin, moxifloxacin, gatifloxacin, levofloxacin, trovafloxacin and grepafloxacin), two macrolides (erythromycin and clarithromycin), as well as tetracycline, against 367 isolates of *C. jejuni* and *C. coli*. Gatifloxacin and moxifloxacin showed the highest activity (MIC_{90} 4 mg/l). Ciprofloxacin had an MIC_{90} of 32 mg/l. This study demonstrated that approximately 38% of the isolates were resistant to at least one fluoroquinolone, with 14.4% simultaneously resistant to all the quinolones. The study also described a marked increase in tetracycline resistance from 22.3% in 1999 to 41.9% in 2004.

3. Surveillance of antimicrobial resistance in campylobacters originating from animals

The monitoring of *Campylobacter* antimicrobial resistance was implemented in France in 1999 for chickens and 2000 for pigs; starting from these years, 200–600 caeca from broilers and pig faecal samples have been collected each year in French slaughterhouses [57,58]. After direct isolation and identification with multiplex PCR, the MIC of ampicillin, nalidixic acid, enrofloxacin or ciprofloxacin, tetracycline, erythromycin and gentamicin was determined by the agar dilution method. For the period 1999–2003, more than 500 *C. jejuni* strains from broilers and more than 1100 *C. coli* from broilers or pigs were analysed. Results (Table 1) indicated that these three populations of strains were inconstantly sensitive to ampicillin, nalidixic acid and fluoroquinolones. A high percentage of resistance to tetracycline was recorded, but all strains remained sensitive to gentamicin. For erythromycin, resistance was rare among *C. jejuni* strains but *C. coli* strains, especially from pig origin, appeared to be frequently resistant.

With regard to poultry production, the percentage of tetracycline-resistant strains was significantly higher when broilers had been treated with oxytetracycline or doxycycline. Moreover, for tetracycline, a significant difference of distribution of resistant strains of *C. coli* was observed between standard and exported broiler productions (81–90% resistant strains) compared to free-range production (51% resistant strains). Similar differences between production types were observed in another study [59], comparing strains isolated from broilers

Table 1
Antimicrobial resistance of *C. jejuni* and *C. coli* from boilers and pigs in France

Antibiotics	MIC^R	*C. jejuni* broilers, % (508[a])	*C. coli* (%)	
			Broilers (296)	Pigs (806)
Ampicillin	16	13–35[b]	25–31	11–15
Nalidixic acid	16	25–44	40–44	19–38
Fluoroquinolones	2	17–32	29–41	11–24
Tetracycline	8	55–68	60–97	83–96
Erythromycin	4	0–5	11–31	48–78
Gentamicin	8	0	0	0

MIC^R: strains were considered resistant when their MIC was superior to MIC^R (mg/l) (Antibiogram Committee of the French Society of Microbiology).
[a] Number of strains studied from 1999 to 2003.
[b] Limits of the annual percentage of resistance.

from 1992 to 1996 and from 2001 to 2002. The percentage of *C. jejuni* strains resistant to ampicillin decreased from 1992–1996 to 2001–2002, whereas no change could be observed for the other antimicrobial agents. However, for *C. coli*, resistance to ampicillin, nalidixic acid, enrofloxacin, tetracycline and erythromycin increased significantly.

3.1. US poultry

The United States is the world's largest producer and exporter of poultry meat, as well as the second-largest egg producer. During 2003, the U.S. poultry meat production totaled 38.5 billion pounds with a total farm value of U.S. poultry production of $23.3 billion (US). Broiler production accounts for the majority of the value at $15.2 billion, followed by eggs at $5.3 billion, turkey at $2.7 billion, and other chicken products at $48 million. Broiler production is concentrated in the southeast, which accounts for over 70% of broilers in the United States wherein broiler production is completed by contract with a grower prior to processing for market. Poultry and egg productions are expected to expand during the future to meet higher domestic and foreign demands for relatively low-cost, healthy meat products due to increased poultry consumption. The Centers for Disease Control and Prevention (CDC) estimates that food-borne diseases cause approximately 76 million illnesses, 325,000 hospitalizations and 5000 deaths in the United States each year with estimates in 2000 for medical costs, productivity losses, and costs of premature deaths that totaled $6.9 billion per year (ERS, USDA; http://www.ers.usda.gov/briefing/poultry/). Among food-borne pathogens active surveillance through FoodNet reports approximately 15 cases is diagnosed each year for each 100,000 persons in the population and campylobacteriosis is estimated to affect over 1 million persons every year, or 0.5% of the general population (CDC; http://www.cdc.gov/ncidod/dbmd/diseaseinfo/Campylobacter_g.htm) with many of these cases associated with consumption of chicken [60].

Although it has been known that isolates may vary in their ability to colonize chicken caeca [61], nearly all broilers at the age of 30–45 days, investigated on Russian poultry farms, were colonized with *Campylobacter* spp. and the level of colonization was on average 10^6–10^7 CFU/g of feces [62], 2004. This high level of *Campylobacter* spp. colonization in broilers was also observed in the USA [63], with 50% of the isolates being both *C. jejuni* and *C. coli* [64]. Carcasses and poultry products contaminated with *Campylobacter* spp. provide a major source for the spread of campylobacteriosis in humans [65] and consumption of commercially prepared chicken is the highest risk factor associated with *Campylobacter* infection in the United States [60]. Although the highest production of poultry is in the southeastern U.S., despite this, even states such as Hawaii have one of the highest rates of *C. jejuni* infections correlated with consuming commercially prepared chicken. Additionally, this high rate of infection was also correlated with prior antibiotic use by affected individuals [66]. It was determined that ciprofloxacin-resistant *Campylobacter* spp. emerged since 1990s, which coincided with the Food and Drug Administration (FDA) approving use of fluoroquinolones in poultry [67] and that 10% of the chicken products purchased from grocery stores contained resistant isolates, with 2% of all isolates resistant to erythromycin [8]. Dramatic increases in isolation of fluoroquinolone-resistant *C. jejuni* have been reported in the U.S. [68], and treatment of chickens with fluoroquinolones can induce rapid selection of ciprofloxacin-resistant campylobacters [69]. Because of the development of antibiotic resistance among food-borne bacterial agents found in poultry, the FDA has proposed discontinued use of enrofloxacin (Baytril TM), which is utilized for the treatment of colibacillosis in chickens and turkeys [70], while sarafloxacin was withdrawn from use during 2001 [71].

In the U.S., poultry litter is spread on fields as a soil amendment for mulch and fertilization in crop improvement, which can be a source of multiple antibiotic-resistant bacteria [72]. This is of concern since *C. jejuni* and *C. coli* can be isolated in nearly equal numbers from chicken and turkeys that carry multiple-drug resistances (tetracycline, doxycycline, erythromycin, nalidixic acid, ciprofloxacin) with co-resistance to ciprofloxacin and erythromycin, found in many *Campylobacter* spp. isolates. Turkey isolates had significantly higher rates of resistance to ciprofloxacin and erythromycin with *C. coli* isolates having significantly higher rates of resistance than *C. jejuni* [73]. In fact, multiple antibiotic-resistant *C. coli* strains apparently predominate among commercial turkey flocks throughout successive production cycles [74]. Fluoroquinolone is not commonly utilized in the U.S. dairy industry, and the isolation of ciprofloxacin-resistant *Campylobacter* spp. is not routine among either conventional or organic dairy farms in the north-central U.S. [75]. Among conventional versus organically raised chickens, both *C. jejuni* and *C. coli* were isolated in approximately 50% of all birds examined, with all the isolates being susceptible to chloramphenicol, but almost 80% were resistant to tetracycline, followed by erythromycin (46%) and ciprofloxacin (8%). Most of the isolates were resistant to ciprofloxacin among the conventionally raised birds than organic chickens, while erythromycin- or tetracycline-resistant isolates were detected in higher amounts among the organically raised birds [76]. During a survey of raw poultry products in the U.S., 84% of the chickens examined were positive for *Campylobacter* spp., and 17% of these isolates were fluoroquinolone-resistant strains that contained the nucleotide substitutions in the *gyr*A gene [77], as previously reported [78]. Finally, class I integron genes associated with potential tobramycin–gentamicin resistance have been detected among *Campylobacter* spp. isolated from U.S. broiler chicken operations [79].

4. Environmental campylobacters and antibiotic resistance

The increasing presence of therapeutic drugs or bacteria resistant to them in the environment highlighted in *Lancet* [80] originates from domestic, farm or hospital wastes [81], before culminating into water or soil environments and is increasingly seen as an ecological problem [82]. Antibiotic resistance

arising from the treatment of clinical and veterinary pathogens has been the focus of recent reviews [5,81]. Recent studies [83] have isolated antibiotic-resistant food-borne pathogens in diverse sources, including farm wastes and their recycled composted products [84,85], incorporating poultry and animal waste elements. An understanding of *Campylobacter* isolates and their antibiotic resistance trends has now emerged as one of the major challenges and a significant problem in both human and animal medicines. For instance, use of fluoroquinolones in poultry production and the emergence of ciprofloxacin-resistant thermophilic campylobacters in humans have been frequently reported [86]. The periodic inventories [4,87] and updates [88] on erythromycin resistance trends on clinically significant isolates of *Campylobacter* from human and food sources further highlight the urgent need for reviewing global variations. Harrow et al [27] defined a baseline frequency of antimicrobial resistance associated with *Campylobacter* isolates from South Canterbury, New Zealand and discussed the likely molecular mechanisms conferring erythromycin resistance in this organism. Together with erythromycin resistance, currently, the contribution to antibiotic resistance in human campylobacteriosis of treating poultry with fluoroquinolones, and the contribution of clinical employment of fluoroquinolones in human medicine is yet to be quantified. A novel method using antibiotic-free medium [89,90] has unravelled environmental *Campylobacter* populations from water and domestic animal faeces samples and their sensitivity to ciprofloxacin. Waldenstrom et al. [91] found a *C. jejuni* isolate resistant to nalidixic acid and ciprofloxacin, indicating that quinolone-resistant genotypes of *C. jejuni* have the potential to spread to wild bird hosts. Griggs et al. [92] isolated quinolone-resistant campylobacters from commercial chicken flocks in high numbers following therapy with a veterinary fluoroquinolone and noted that the prevalence of resistant campylobacters in the faeces of some flocks prior to slaughter may have consequences for public health. Previous research data [72] and recent investigations [93] demonstrate unequivocally, how microbial contamination of litter should be reduced or eliminated prior to re-utilization (e.g. production of compost based-fertilizers) to minimize environmental health risks related to transfer of antibiotic-resistant bacteria to humans or other animals. Santamaria and Toranzos [94] have comprehensively reviewed the implications in over two decades of waste disposal practices into soil and the fate of enteric pathogens including campylobacters. Hutchison et al. [83] showed that not incorporating contaminated livestock wastes into soil is a potential intervention measure that may help to limit the spread of zoonotic agents further up the food chain. Hutchison et al. [95] recently surveyed the levels and prevalence of zoonotic agents in 1549 livestock waste samples and their significance with livestock husbandry and farm waste management practices and showed that statistically significant livestock groups containing calves of less than 3 months of age, piglets, or lambs had higher prevalence and levels of *Campylobacter* spp. and *E. coli* O157 in their wastes. Shea [96] has recently reviewed the dangers of non-therapeutic use of antimicrobials in animal-agriculture. In view of the implications arising from the drugs used in animal-foods bearing similarity to the antibiotics in humans, the United States Public Health and the US Academy of Pediatrics are jointly disseminating policies on the judicious use of antimicrobial agents.

5. Biocide resistance

Although campylobacters are mainly food-borne, water is also regarded as an important route for the transmission of these pathogens [97]. In the developed world, water quality regulations require that potable water does not contain any microbial pathogens [98]. While this is generally achieved, the incidence of water-borne outbreaks associated with potable water has increased over the past decade [98]. Water plays an important role in the ecology of *C. jejuni* [99], which can enter drinking water distribution systems through faecal contamination of untreated ground or surface water, treatment failure or distribution system failure (interconnections with contaminated waste water collection systems) [100]. The standard chlorination procedures are sufficient to prevent the spread of planktonic campylobacters along water mains, if the water is of low turbidity [100,101]. One of the main risk incidents in water treatment is associated disinfection malfunction [102]. Many factors including chemical category and formulation, concentration, exposure time, presence of organic matter, type and quantity of microorganisms, temperature, pH and water hardness can modify disinfectant effectiveness [103,104].

No link between resistance to disinfectants and antibiotics has been observed [103]. To ensure the effectiveness of disinfectants, an exposure time recommended by manufacturers is generally longer than 5 min [103]. Ethyl alcohol and iodophor compounds are commonly used in hospitals to disinfect skin and thermometers, and their recommended concentrations are effective against *C. jejuni* [104]. Although nosocomial infections due to the misuse of quaternary ammonium compounds have been reported, these compounds are widely employed for disinfection in hospitals and laboratories at recommended dilutions of 1:500 effectively kill *C. jejuni* in 1 min [104]. Formalin (10%) is commonly used to fix and disinfect tissues, and effectively kills planktonic *C. jejuni* [104].

Similar concentrations of hypochlorite are required to kill equal densities of *E. coli* and *C. jejuni* [104]. An inoculum of 10^4 CFU/ml, 1.25 mg of hypochlorite per liter generally kills *C. jejuni* within 1 min. Levels of chlorination normally found in potable waters would normally be considered lethal to planktonic *C. jejuni* [105,106]. In aquatic environments, disinfectants might be less effective due to the interference between *Campylobacter* or disinfectant and organic matter [103], and interactions with water-borne protozoa [106]. Water-borne protozoa, such as *Acanthamoeba castellanii* and *Tetrahymena pyriformis*, have the potential to act as protective reservoirs for *C. jejuni* in the drinking water systems of intensively reared broilers [106]. Experimental co-cultivation of *C. jejuni* with such protozoa can significantly reduce the susceptibility of the bacteria to chlorine [105], as well as to the

industrial disinfectant Virudene ($p < 0.05$) [106]. These factors may partially explain observations by Stern et al. [107] that chlorination of broiler drinking water had no effect on the *C. jejuni* colonization of broilers. Water-borne protozoa could also provide protective environments for transferring antibiotic resistance genes, which could then be exchanged between strains. In stress conditions, e.g. a nutrient poor aqueous environment, *Campylobacter* can adopt a viable but non-cultivable form (VBNC). No evidence has shown the existence of VBNC *Campylobacter* forms after treatment with disinfectants [103]. Experimental evidence suggests that during water-related environmental stresses *C. jejuni* generates forms that are VBNC, especially in the presence of biofilms [65]. The survival of such forms for periods of up to 4 months has been recorded [65], but survival times appear to vary with water temperature, strain, the aquatic system used, and previous growth conditions [65]. Biofilms promote survival through a variety of mechanisms, e.g. uptake by protozoa [108], and their presence enhances the survival of culturable and VBNC forms of *C. jejuni* [109] and also increases disinfection resistance [110].

6. Future prospects

The past 10 years have allowed the elucidation of several antibiotic resistance mechanisms in the thermophilic campylobacters. By combining such data along with conventional and molecular epidemiological information, we are now beginning to gain a better understanding of how antibiotic resistance is initiated and acquired in organisms and how such organisms may be transmitted to new animal and human hosts. Globally, antibiotic resistance rates are beginning to rise against several antibiotic agents and indeed, multiple resistance patterns to several classes of antibiotics, are beginning to emerge. Although unlike certain other Gram-negative organisms, such as *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, where multi-resistance has in certain isolates now fulminated into pan-resistance, we have not reached this point in the ecological succession of antibiotic resistance in the campylobacters. Several agents still remain extremely effective against the vast majority of the campylobacters, including gentamicin; however, with such agents, the lack of an oral preparation will limit the employment of such agents. Therefore, clinical medicine still has a number of effective agents which can be relied upon to treat infections due to these organisms.

As a race, there is responsibility on humanity to treat sick animals as well as sick humans. Problems may therefore arise when sick animals are colonized and indeed are infected with similar organisms that have the potential to cause mild to serious diseases in humans. Given that there is a finite number of effective therapeutic agents that can be used to successfully treat infections in both animal and human populations, combined with the fact that antibiotic resistance is increasing at a faster rate than new antibiotic agents are being developed and released onto the market, we have to employ great prudence in deciding appropriate and fair mechanisms as to how antibiotics can be shared, so as to obtain the optimal effect for humans, as well as animals. Collective responsibility between the major stakeholders in this arena, including farmers, veterinary surgeons, food manufacturers, clinicians and consumers, is required at all local and international levels and a workable mechanism developed, so as to avoid further problems associated with acquiring antibiotic resistance with the campylobacters.

A continued vigilant approach is required, at a local, national and international level, with enhanced surveillance and reporting of trends, particularly if increased resistance rates are being demonstrated, so that appropriate control and intervention measures may be put in place to limit the emergence of resistance problems in this, still important, organism of major public health significance.

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